Urol Res (2005) 33: 349–357
DOI 10.1007/s00240-005-0492-4

INVITED REVIEW

Saeed R. Khan

# Hyperoxaluria-induced oxidative stress and antioxidants for renal protection

Received: 7 June 2005 / Accepted: 8 June 2005 / Published online: 15 November 2005


**Abstract** Renal cellular exposure to oxalate (Ox) and/or CaOx crystals leads to the production of reactive oxygen species (ROS), development of oxidative stress followed by injury and inflammation. Renal injury and inflammation appear to play a significant role in stone formation. ROS are produced from many sources and involve a variety of signaling pathways. Tissue culture and animal model studies show that treatments with anti-oxidants and free radical scavengers reduce Ox/CaOx crystal induced injuries. In addition, CaOx crystal deposition in kidneys is significantly reduced by treatments with antioxidants and free radical scavengers, indicating their efficacy. These results point towards a great potential for the therapeutic application of antioxidants and free radical scavengers to reduce stone recurrence particularly after shock wave lithotripsy, which is itself known to generate ROS and cause renal damage.

**Keywords** Reactive oxygen species · Oxidative stress · Oxalate · Calcium oxalate · Nephrolithiasis

## Introduction

Oxalate is a natural byproduct of metabolism and in normal individuals is harmlessly excreted. However, increased urinary excretion of oxalate (Ox), hyperoxaluria, can be toxic largely because of its propensity to crystallize at physiologic pH and form calcium oxalate (CaOx) crystal deposits in the kidneys [1]. In the kidneys, CaOx crystals can block the renal tubules, disrupt cellular functions and kill nearby cells. Hyperoxaluria is a result of either genetic (primary hyperoxaluria) or environmental factors (secondary hyperoxaluria). Enhanced absorption of Ox, secondary to many gastrointestinal diseases, ileal resection, or jejuno-ileal bypass is called enteric hyperoxaluria [2]. Even though various hyperoxalurias have distinct origins, the pathologies they induce can often be indistinguishable, encompassing urolithiasis, nephrocalcinosis, metabolic acidosis, hematuria, pyelonephritis, hydronephrosis and renal failure. In the case of primary hyperoxaluria, there is a systemic deposition of CaOx in almost all of the body tissues including kidneys, heart, bone, cartilage, teeth, vasculature and brain. Patients with primary hyperoxaluria eventually develop end-stage renal failure, usually in childhood. Patients with enteric hyperoxaluria may also develop renal inflammation and end stage renal disease.

Recent research has shown that the response of renal epithelial cells to Ox and CaOx crystals is biphasic and concentration dependent [3–5]. Ox by itself is mitogenic at low concentrations and toxic at higher concentrations as well as in association with CaOx crystals. Injury to the renal epithelial cells results in cellular degradation and the production of membranous vesicles [6]. The crystals are either passed as crystalluria particles or are endocytosed by the epithelial cells to be processed by their lysosomal system or transported to the interstitium. CaOx crystal deposition in the kidneys upregulates the expression and/or synthesis of macromolecules, which can promote inflammation and lead to fibrosis [7, 8]. In animals and renal epithelial cells in culture, reaction to high Ox and CaOx crystals is associated with the generation of free radicals [9, 10]. Antioxidants reduce hyperoxaluria and CaOx crystal induced toxicity [11, 12]. This article discusses free radicals and reactive oxygen species (ROS), their role in the activation of Ox and CaOx induced signaling pathways and triggering of renal injury, and their control by antioxidant treatment.

S. R. Khan
Box 100275–0275, Department of Pathology,
Immunology and Laboratory Medicine,
University of Florida, Gainesville Florida 32610, USA
E-mail: khan@pathology.ufl.edu
Tel.: +1-352-392-3574
Fax: +1-352-3923053

## Free radicals and reactive oxygen species

Free radicals, atoms or molecules with unpaired electrons, and their metabolites, collectively called reactive oxygen species (ROS) are highly reactive, playing a critical role as signaling molecules. However, they can also produce chemical modifications of, and damage to, proteins, lipids, carbohydrates and nucleotides [13, 14]. Major cellular ROS include the superoxide anion ($O_2^{-\cdot}$), nitric oxide radical ($NO^{\cdot}$), hydroxyl radical ($OH^{\cdot}$), and hydrogen peroxide ($H_2O_2$), which are generated by several pathways. $O_2^{-\cdot}$ anions are produced by NADPH oxidases, xanthine oxidase, lipooxigenase, cyclooxygenase, hemeoxygenase and as a byproduct of the mitochondrial respiratory chain. Lipid radicals can also produce $O_2^{-\cdot}$. $NO^{\cdot}$ radicals are produced by the endothelial nitric oxide synthase (eNOS) mediated oxidation of L-arginine. eNOS can also produce $O_2^{-\cdot}$ rather than NO. Reactions between superoxide and nitric oxide can produce the highly reactive peroxynitrite $ONOO^{-}$.

Cells are equipped with a number of scavenging systems to limit ROS. These include superoxide dismutase (SOD) to eliminate $O_2^{-\cdot}$, and glutathione (GSH) peroxidase and catalase to detoxify $H_2O_2$ (Fig. 1). Superoxide has a short half-life and spontaneously converts to $H_2O_2$. The reaction is noticeably enhanced by SOD. $H_2O_2$ is long-lasting and far more reactive than superoxide anions. Moreover, in a more complex transition metal catalyzed reaction called the metal catalyzed Haber-Weiss reaction, $H_2O_2$ yields an even more reactive hydroxyl radical, which is, however, short lived and works at short range. Initially, superoxide anions donate single electrons to ferric ions resulting in molecular oxygen and ferrous ions. Reaction between ferrous ions and $H_2O_2$, the so-called Fenton reaction, leads to the formation of $OH^{\cdot}$. $H_2O_2$ is subsequently metabolized to water via catalase or by glutathione peroxidase in the presence of reduced glutathione.

Under normal conditions the superoxide anions ($O_2^{-\cdot}$), NO radicals ($NO^{\cdot}$) and their metabolites are generated by tightly controlled enzymes and serve as mediators in a variety of regulatory processes and signaling pathways including proliferation, activation or inactivation of regulatory biomolecules, and regulation of transcriptional activities. Signaling molecules regulated by ROS include, but are not limited to, protein tyrosine kinases and phosphatases, serine/theorine kinases and phosphatases, Ras, various phospholipases, and many calcium signals. ROS also regulate such genes as *c-fos, c-myc, and c-jun* and transcription factor activation protein-1 (AP-1) and nuclear factor κB (NF-κB). In addition, ROS also participate in the initiation and implementation of apoptosis. Evidence also exists for cross talk between cellular signaling and ROS production.

Since ROS and reactive nitrogen species (RNS) play significant regulatory roles, they normally occur at steady state levels, generated when needed and then cleared by activities of various antioxidants and scavengers. But uncontrolled generation of the reactive oxygen or nitrogen species and/or a reduction in the endogenous antioxidant capacity creates oxidative stress. Most cells respond to oxidative stress by boosting the levels of intracellular antioxidants such as glutathione. The oxidants can react with all the basic constituents of cells: lipids, carbohydrates, proteins and nucleic acids severely affecting their structure and function. The pathological changes may result from the damaging effects of ROS and from ROS-mediated changes in gene expression and signal transduction.

## Hyperoxaluria-induced oxidative stress

An overproduction of ROS and/or a reduction in cellular antioxidant capacities, due to down-regulated expression of the antioxidant enzymes, leads to the development of oxidative stress (OS). In view of the fact that most ROS are short-lived and do not travel long distances, the presence of OS is generally recognized by abundance of byproducts of ROS interaction with lipids, amino acids, proteins, carbohydrates and nucleic acids. Malondialdehyde, isoprostanes, and oxidized lipids are among the most common byproducts of ROS induced OS.

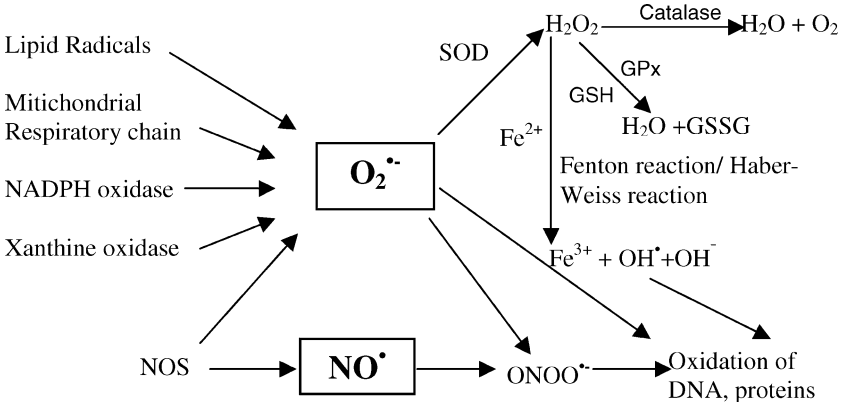


**Fig. 1** Reactive oxygen species. Sources and reactions involved in the production of superoxide ($O_2^{-\cdot}$), nitric oxide ($NO^{\cdot}$), hydrogen peroxide ($H_2O_2$), peroxynitrie ($ONOO^{-}$), and hydroxyl radicals ($OH^{\cdot}$). *GSH*, glutathione; *GPx*, glutathione peroxidase; *GSGG*, oxidised glutathione; *NOS* nitric oxide synthase [13, 14]

## Evidence from clinical studies

Recent studies have provided evidence for the development of OS in the kidneys of stone patients. Results of one such study showed that CaOx kidney stone patients excrete significantly higher amounts of α-glutathione S-transferase (α-GST), malondialdehyde (MDA) and thiobarbituric acid-reactive substances (TBARS) in their urine, indicating OS in kidneys of CaOx stone patients [15]. In addition, urinary excretion of β-galactosidase (GAL) and N-acetyl-β-glucosaminidase (NAG) were also significantly increased in stone patients. Urinary excretion of these enzymes is considered a marker of renal epithelial injury. Similarly, another recent study showed that stone disease is associated with high oxidative stress and renal tubular cell damage [16]. Compared to the normal controls, stone patients had higher plasma MDA, urinary MDA, and higher urinary NAG activity, but lower reduced glutathione (GSH) and cellular glutathione peroxidase (cGPx) activity, protein thiol and vitamin E.

## Evidence from tissue culture studies

Evidence for the involvement of ROS in hyperoxaluria-induced OS stress and cell injury originally came from tissue culture studies in which renal epithelial cells were exposed to Ox and/or CaOx crystals. Exposure of both LLC-PK1 and MDCK cells, representing the proximal tubular and collecting duct epithelium, respectively, to Ox and CaOx crystals, induced the production of superoxide as well as $H_2O_2$, and was associated with decreased trypan blue exclusion, and increased activities of LDH, GGTP, and NAG in the culture media [9, 11, 12]. Antioxidants vitamin E, catalase and desferoxamine (DFO), superoxide dismutase (SOD), and its mimetic provided protection from the generation of ROS and associated lipid peroxidation and injury. Treatment with antioxidants significantly reduced the release of LDH and the formation of malondialdehyde and restored the cellular antioxidant enzymes glutathione peroxidase and catalase to normal levels.

Cellular GSH provides a major defense against oxidative injury. The exposure of MDCK cells to Ox or/and COM crystals decreased total glutathione and led to a large increase in the concentration of NADP/NADPH [17]. Exposure of LLC-PK1 cells to Ox significantly increased cellular ceramides [18]. Pretreatment with glutathione precursor N-acetylcysteine (NAC) blocked this Ox-induced increase in ceramide levels.

Citrate is also involved in maintaining endogenous antioxidant defenses. Administration of exogenous citrate to LLC-PK1 and MDCK cells bolstered these defenses and diminished the cellular injury inflicted by exposure to increased Ox and CaOx crystals [19]. Presence of citrate in the culture medium was associated with a significant increase in glutathione (GSH) and a drop in the production of $H_2O_2$ and 8-isoprostane (8-IP), an end product of lipid breakdown. There was a significant improvement in cell viability as shown by significantly decreased LDH release and increased trypan blue exclusion.

## Evidence from animal model studies

Results of animal model studies have confirmed the production of reactive oxygen species and changes in renal cellular endogenous antioxidant defenses leading to hyperoxaluria-induced oxidative stress. Rats with hyperoxaluria and CaOx nephrolithiasis induced by ethylene glycol showed increased urinary excretion of lipid peroxides and LDH [10]. In addition, administration of antioxidant vitamin E resulted in reduced lipid peroxides in the kidneys and decreased urinary excretion of LDH and lipid peroxides. This points to ROS involvement in Ox and CaOx crystal associated renal toxicity. Deposition of CaOx crystals in the kidneys was associated with a reduction in total renal cellular glutathione and an increase in lipid peroxides [20]. Rats which received the angiotensin converting enzyme (ACE) inhibitor losartan, known to reduce oxidative stress, showed a significant increase in glutathione concentration and a decrease in the thiobarbituric acid reactive substances in the kidneys. Activities of catalase and MnSOD increased in kidneys while α- and μ-glutathione-S-transferase (GST) levels increased in the urine of hyperoxaluric rats [21].

Microarray analysis of the kidneys of hyperoxaluric rats also revealed the development of oxidative stress during hyperoxaluria and CaOx crystal deposition. Expression of genes for SOD, GPx, GST, aldehyde

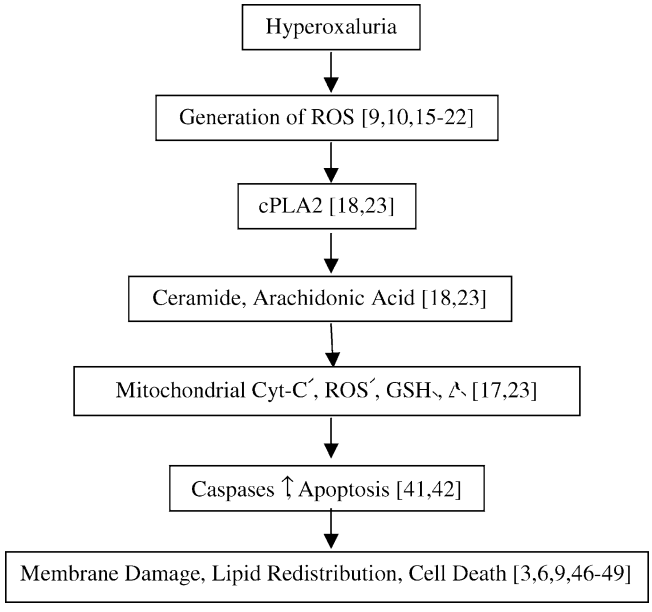


**Fig. 2** Mitochondrial involvement in hyperoxaluria-induced cell injury

dehydrogenase, mitochondrial uncoupling protein and ceruloplasmin was increased in hyperoxaluric rats [22].

## Sources of reactive oxygen species in hyperoxaluria—induced oxidative stress

Mitochondria are generally the most common source of superoxide and $H_2O_2$ in most cells and tissues (Fig. 2). Selective probes, substrates and inhibitors show the mitochondria to be the major site of CaOx crystal induced superoxide production and glutathione depletion in both LLC-PK1 and MDCK cells [17]. In vivo CaOx crystal deposition in the kidneys correlates with depletion of mitochondrial glutathione [20]. Furthermore, Ox exposure can cause a decrease in mitochondrial membrane potential in MDCK cells. Isolated mitochondria responded to Ox exposure by the accumulation of ROS, lipid peroxides and oxidized thiol proteins [23]. Therefore, mitochondrial changes can be the result of Ox-induced PLA2 activation and release of lipid signaling molecules.

Another likely source of Ox induced superoxide production is membrane-associated NADPH oxidase, which is also a major source of ROS in the kidneys [24, 25], particularly in the presence of angiotensin II [26, 27]. Angiotensin II is implicated in causing oxidative stress by stimulating membrane bound NAD(P)H oxidase leading to increased generation of superoxide [28]. A significant reduction in hyperoxaluria-induced production of renal lipid peroxides after administration of AT 1 receptor blockers [20, 29] or ACE inhibitors [30] was seen in hyperoxaluric rats. In addition these treatments produced a reduction in TGF-$\beta$ expression in the kidneys. TGF-$\beta$ has been shown to participate in ROS production through the activation of NADPH oxidase.

Tissue culture studies have indicated involvement of NADPH oxidase in Ox-induced ROS production and cell injury. Ox-induced injury of NRK52E cells was significantly reduced in the presence of diphenyleneiodonium chloride (DPI), an NADPH oxidase inhibitor [31–33]. Ox-associated activation of NADPH oxidase in LLC-PK1 cells was associated with increased production of TGF-$\beta_1$. Treatment with neutralizing TGF-$\beta$ antibodies significantly reduced the generation of ROS [33].

## Signaling pathways involved in hyperoxaluria-induced ROS generation

Since many signaling pathways are activated by ROS [13, 14], another approach to study their generation during hyperoxaluria is to investigate the regulation of these pathways. Figure 3 shows some of the pathways involved. ROS activate signaling molecules such as protein kinase C (PKC), c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK), and transcription factors such as NF-$\kappa$B and activated protein-1 (AP-1). Activation of these molecules leads to upregulation of genes and proteins such as monocyte chemoattractant protein-1 (MCP-1), osteopontin (OPN), fibronectin and TGF-$\beta$1. Both Ox and CaOx

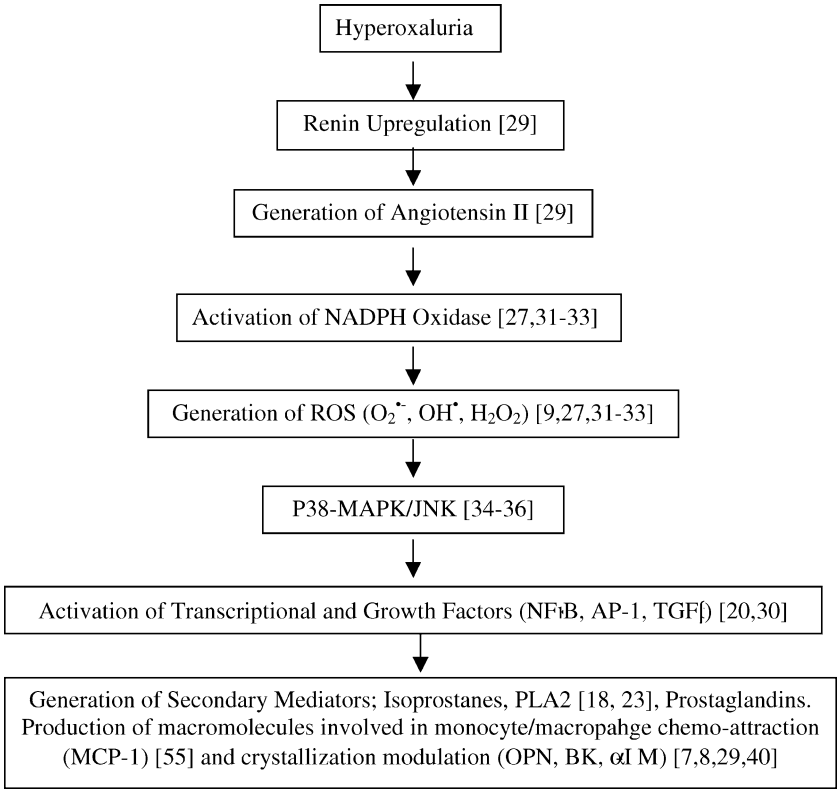


**Fig. 3** Schematics of hyperoxaluria-induced generation of reactive oxygen species (ROS) and their effect on cellular physiology and pathology. $O_2^{\cdot-}$, superoxide; $OH^{\cdot}$, hydroxyl radical; $H_2O_2$, hydrogen peroxide; *OPN*, osteopontin; *BK*, bikunin; *MCP-1*, monocyte chemoattractant protein-1

crystals selectively activated p38 MAPK (mitogen activated protein kinase) signal transduction pathways [34, 35] in the proximal tubular epithelial cells. Activation of p38 MAPK was found essential for the reinitiation of Ox-induced DNA synthesis. Ox exposure also caused modest activation of JNK as determined by c-Jun phosphorylation. Ox-induced cell proliferation was prevented by antioxidant N-acetyl-L-cysteine (NAC), a p38 MAPK inhibitor, a SAPK/JNK (stress activated protein kinase/c-Jun NH2-terminal kinase) inhibitor as well as PLA2 inhibitors indicating the involvement of ROS mediated signal transduction [36]. Apparently, a renal epithelial response to Ox involves signal transduction via MAP kinases, similar to the cellular response to many other challenges.

Cytosolic phospholipase $A_2$ ($cPLA_2$) is released upon the activation of MAP kinases and translocated to the cell membrane. $cPLA_2$ preferentially hydrolyses arachidonoyl phospholipids generating a number of byproducts including arachidonic acid and lysophospholipids. Exposure of MDCK cells to Ox produces a time and concentration dependent increase in $cPLA_2$ activity [37]. Inhibition of $cPLA_2$ activity blocked the Ox-induced upregulation of *Egr-1*, *c-jun* and *c-myc* genes. Exposure of LLC-PK1 as well as MDCK cells to Ox also increased the generation and accumulation of ceramide, another signaling lipid, most probably through the activation of neutral sphingomyelinase. An inhibitor of cytoplasmic PLA2, as well as pretreatment with NAC and a superoxide dismutase mimetic blocked ceramide production, indicating the involvement of ROS signaling and PLA2 pathway [18, 37].

Animal model studies have provided evidence for the hyperoxaluria-induced activation of the renin-angiotensin system [20, 29, 30], a major player in renal disease progression [38]. Hyperoxaluria induced oxidative stress, inflammation, fibrosis and CaOx crystal deposition in kidneys of rats was significantly reduced by the administration of ACE inhibitor [30] and angiotensin II type 1 (AT1) receptor blockade [20, 30]. In our study [29], a group of hyperoxaluric rats was treated with AT1 receptor blocker, candesartan. At the end of 4 weeks, mRNA for OPN, renin and ACE, as well as MDA were significantly elevated in the kidneys of hyperoxaluric rats. The kidneys of hyperoxaluric rats on candesartan had fewer CaOx crystal deposits, fewer interstitial ED1 positive cells, reduced OPN expression and reduced MDA than hyperoxaluric rats. Kidneys produce both angiotensinogen and ACE, and the juxtaglomerular apparatus is the main source of circulating renin. Renin catalyzes the production of angiotensin I which is converted to Ang II by the actions of ACE. Ang II acts through two receptors, types 1 (AT1) and 2 (AT2), and mediates many activities of the renin-angiotensin system. It is a major pro-inflammatory molecule. Oxidative stress plays a significant role in Ang II operations [13, 14, 38].

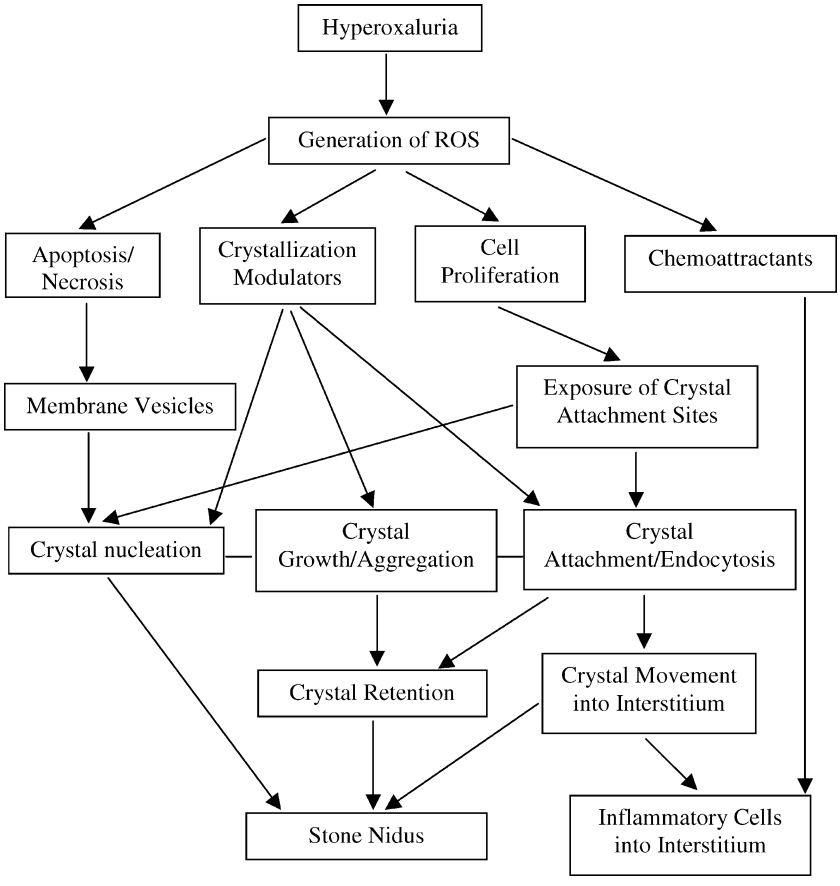


**Fig. 4** Hyperoxaluria induced generation of reactive oxygen species and their effect on various aspects of crystal formation and retention within the kidneys leading to stone formation

Calcium antagonists are known to reduce ROS production, the development of oxidative stress and the progression of various renal diseases. Administration of amlodipine, a dihydropyridine-based calcium antagonist, reduced hyperoxaluria-induced inflammation, fibrosis and crystal deposition in the rat kidneys [39].

## Oxidative stress and CaOx nephrolithiasis

High concentrations of Ox and CaOx crystals also provoke renal cells to increase the synthesis of various mediators of the inflammatory processes and extracellular matrix production, and modulators of crystallization [7, 8, 40]. Reactive oxygen species are involved in the activation of signaling molecules such as protein kinase C (PKC), c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK), with influence over transcription factors such as NF-$\kappa$B and activated protein-1 (AP-1). Activation of these transcription factors leads to upregulation of genes and production of crystallization modulators such as OPN, bikunin, and $\alpha$-1-microglobulin [7, 8, 40], which affect all aspects of nephrolithiasis including crystal formation, growth, aggregation as well as their retention within the kidneys.

Oxidative stress is injurious to all components of the cells. How does cell injury caused by hyperoxaluria-induced oxidative stress promote nephrolithiasis? Figure 4 outlines the lithogenic effects of hyperoxaluria-induced generation of ROS. Previous studies from many laboratories including our own indicate that damage to the urothelium may predispose to de novo crystallization and crystal retention in the kidneys. We have demonstrated that experimentally induced hyperoxaluria in rats results in renal tubular cell damage and CaOx crystal deposition [6]. Both apoptotic and necrotic injuries have been detected [41, 42]. Crystals always deposit at sites of tubular injury in association with membrane vesicles and are also seen attached to the exposed basement membrane [6, 43, 44]. Our in vitro studies have shown that membranes and lipids of cellular degradation products are excellent nucleators of CaOx crystals at supersaturation normally found in the renal tubular fluids [45]. Tissue culture studies in which renal epithelial cells were exposed to Ox and/or CaOx crystals have shown that epithelial injury promotes attachment of CaOx crystals [46, 47]. This attachment is mediated by Ox-induced exposure of phosphatidylserine (PS) on cell surfaces [48, 49]. Interestingly, apoptosis involves the exposure of PS on cell surfaces [41].

## Antioxidants for renal protection

Figure 5 illustrates the stages in which specific antioxidants and other protective detoxification treatments have been shown to impede the development of hyperoxaluria-induced oxidative stress. Pretreatment with vitamin E ($\alpha$-tocopherol) along with mannitol abolished the deposition of CaOx crystals in the kidneys of rats injected with sodium oxalate [50]. Alanine-induced deposition of CaOx crystals in rat kidneys was blocked by dietary supplementation with vitamin E plus selenium [51]. Interestingly, vitamin E alone caused only a decrease in crystal deposition, while combined treatments totally abolished it. Treatment with methionine [52] or glutathione monoester [53] also reduced renal CaOx crystal deposits in the kidneys of hyperoxaluric rats. The reduction or total elimination of crystal deposition was associated with restoration of the anti-oxidation defenses of the kidneys by increasing activities of the enzymes SOD, catalase, glutathione peroxidase (GPx) and/or free radical scavengers, reduced glutathione (GSH), ascorbic acid, vitamin E and protein thiol groups. Vitamin E is the major lipid soluble antioxidant present in the cell membranes and acts synergistically with the other antioxi-

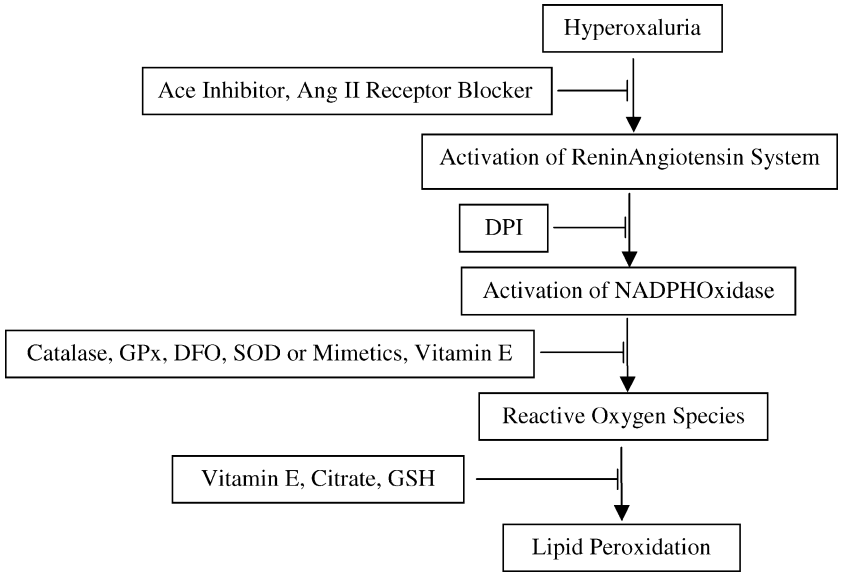


**Fig. 5** Antioxidant and other detoxification treatments to impede the development of hyperoxaluria induced oxidative stress

dants. It can react with lipid radicals and stop the propagation of lipid peroxidation. Selenium is normally incorporated in GPx. Mannitol is a scavenger of hydroxyl radicals while methionine is a thiol generating compound.

Green tea has recently been shown to reduce CaOx crystal deposits in the kidneys of rats made hyperoxaluric by the administration of ethylene glycol [54]. Reduced crystal deposition was coupled with improved SOD activity. In addition, green tea consumption caused a decrease in apoptotic activity in the kidneys. It was concluded that antioxidants, catechins, present in green tea were mainly responsible for these improvements.

Reduction of angiotensin production by inhibiting ACE or blocking angiotensin receptors has been shown to significantly reduce renal CaOx crystal deposition as well as the development of interstitial inflammation [20, 30, 38]. These treatments also resulted in a reduction in the oxidative stress measured as products of lipid peroxidation. Angiotensin II is implicated in causing oxidative stress by activating membrane associated NADPH oxidase, which leads to the production of superoxide [38]. ROS generated through the activation of NADPH oxidase are also involved in the production of MCP-1, for the recruitment of monocytes/macropahges to the interstitium. Exposure of renal epithelial cells in culture to Ox caused ROS dependent upregulation of the MCP-1 gene, and the production and secretion of the protein [55].

Antioxidant treatment for stone disease has not been clinically tested. However, some well-known antioxidants such as citrate and allopurinol, have been evaluated in a number of prospectively randomized trials, and their efficacy has been established [56] in populations of recurrent stone formers. Citrate increases cellular nicotinamide adenine dinucleotide phosphate and reduced glutathione while allopurinol is a xanthine oxidase inhibitor.

## Conclusions

There is plenty of experimental and clinical indication for the production of ROS, development of oxidative stress and associated cellular injury when renal cells are exposed to Ox and/or CaOx crystals. In addition, antioxidant treatments reduce CaOx crystal deposition in the kidneys of experimental animals providing proof of the principle of the efficacy of antioxidants. Therefore it is advisable to evaluate the therapeutic application of antioxidants on reducing stone recurrence particularly after shock wave lithotripsy (SWL) since it is itself known to generate ROS and cause renal damage. Because the development of oxidative stress involves many sources and a variety of signaling pathways, it is essential to identify all enzymes and pathways active in Ox and CaOx crystal induced generation of ROS. A combination of anti-oxidants and free radical scavengers may provide superior renal protection.

In a number of cardiovascular diseases including hypertension, type II diabetes, hyperlipidemia, atherosclerosis and chronic heart failure, in which ROS play major role in disease development, lowering oxidative stress is considered a proper therapeutic approach [57, 58]. Observational data in humans indicate that antioxidant vitamin E intake is associated with reduced cardiovascular disease [59, 60]. Safety of vitamin E up to doses of 800 IU/day has been established. However, the prospective, controlled clinical trials of vitamin E present a complicated picture. Reasons for this discrepancy include inappropriate endpoints and patients and ineffective antioxidants. Most trials have tested the efficacy of vitamins E and C [58], however, both can become pro-oxidant, particularly at higher concentrations. In addition, vitamin E is not a very efficient antioxidant. The rate constant for reaction between superoxide and vitamin E is low and its tissue concentration does not reach high enough levels for biological effects. It is important to point out that vitamin E, given alone, only partially reduced the oxidative stress and CaOx crystal deposition in rat kidneys. Vitamin C has an added disadvantage as a treatment for nephrolithiasis, in that it may increase urinary excretion of Ox [61].

## References


1. Danpure CJ, Purdue PE (1995) Primary hyperoxaluria. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease, 7th edn. McGraw-Hill, New York, p 2385
2. Hatch M, Freel RW, Vaziri ND (1999) Regulatory aspects of oxalate excretion in enteric oxalate elimination. J Am Soc Nephrol 10: S324
3. Hackett RL, Shevock PN, Khan SR (1994) Madin-Darby canine kidney cells are injured by exposure to oxalate and to calcium oxalate crystals. Urol Res 22: 197
4. Koul H, Kenington L, Nair G, Honeyman T, Menon M, Scheid CR (1994) Ox-induced initiation of DNA synthesis in LLC-PK1 cells, a line of renal epithelial cells. Biochem Biophys Res Commun 205: 1632
5. Koul H, Kenington L, Honeyman T, Jonassen J, Menon M, Scheid CR (1996) Activation of the c-myc gene mediates the mitogenic effects of Ox in LLC-PK1 cells, a line of renal epithelial cells. Kidney Int 50: 1525
6. Khan SR (1995) Calcium oxalate crystal interaction with renal tubular epithelium, mechanism of crystal adhesion and its impact on stone development. Urol Res 23: 71
7. Khan SR (2004) Crystal-induced inflammation of the kidneys: results from human studies, animal models, and tissue culture studies. Clin Exp Nephrol 8: 75
8. Khan SR (2004) Role of renal epithelial cells in the initiation of calcium oxalate stones. Nephron Exp Nephrol 98: 55
9. Scheid CR, Koul H, Hill WA, Luber-Narod J, Kennington L, Honeyman T, Jonassen J, Menon M (1996) Ox toxicity in LLC-PK1 cells: role of free radicals. Kidney Int 49: 413
10. Thamilselvan S, Hackett RL, Khan SR (1997) Lipid peroxidation in ethylene glycol induced hyperoxaluria and CaOx nephrolithiasis. J Urol 157: 1059
11. Thamilselvan S, Hackett RL, Khan SR (2000) Free radical scavengers catalase and superoxide dismutase provide protection from oxalate associated injury to LLC-PK1 and MDCK cells. J Urol 164: 224

12. Thamilselvan S, Khan SR, Menon M (2003) Oxalate and calcium oxalate mediated free radical toxicity in renal epithelial cells: effect of antioxidants. Urol Res 31: 3
13. Drodge W (2001) Free radicals in the physiological control of cell function. Physiol Rev 82: 47
14. Kamata H, Hirata H (1999) Redox regulation of cellular signalling. Cell Signal 11: 1
15. Huang H-S, Ma M-C, Chen C-F, Chen J (2003) Lipid peroxidation and its correlations with urinary levels of oxalate, citric acid, and osteopontin in patients with renal calcium oxalate stones. Urology 61: 1123
16. Tungsanga K, Sriboonlue P, Futrakul P, Yachantha C, Tosukhowong P (2005) Renal tubular cell damage and oxidative stress in renal stone patients and the effect of potassium citrate treatment. Urol Res (in press)
17. Khand FD, Gordge MP, Robertson WG, Noronha-Dutra AA, Hothersall JS (2002) Mitochondrial superoxide production during oxalate mediated oxidative stress in renal epithelial cells. Free Radic Biol Med 32: 1339
18. Cao L-C, Honeyman T, Jonassen J, Scheid C (2000) Oxalate-induced ceramide accumulation in Madin-Darby canine kidney and LLC-PK1 cells. Kidney Int 57: 2403
19. Byer K, Khan SR (2005) Citrate provides protection against oxalate and calcium oxalate crystal induced oxidative damage to renal epithelium. J Urol 173: 640
20. Toblli JE, Ferder L, Stella I, De Cavanagh MVE, Angerosa M, Inserra F (2002) Effects of angiotensin II subtype 1 receptor blockade by losartan on tubulointerstitial lesions caused by hyperoxaluria. J Urol 168: 1550
21. Huang H-S, Ma M-C, Chen J, Chen C-F (2002) Changes in the oxidant-antioxidant balance in the kidneys of rats with nephrolithiasis induced by ethylene glycol. J Urol 167: 2584
22. Chen D H-C, Kaung H-LC, Miller CM, Resnick MI, Marengo SR (2004) Microarray analysis of changes in renal phenotype in the ethylene glycol rat model of urolithiasis: potential and pitfalls. BJU Int 94: 637
23. Cao L-C, Honeyman T, Cooney R, Kennington L, Scheid C R, Jonassen J A, (2004) Mitochondrial dysfunction is a primary event in renal cell oxalate toxicity. Kidney Int 66: 1890
24. Li N, Yi FX, Spurrier JL, Bobrowitz CA, Zou AP (2002) Production of superoxide through NADH oxidase in thick ascending limb of the loop in rat kidney, Am J Physiol 282: F1111
25. Geiszt M, Kopp JB, Varnai P, Leto TL (2000) Identification of renox, an NAD(P)H oxidase in kidney. Proc Natl Acad Sci U S A 97: 8010
26. Hanna IR, Taniyama Y, Szocs K, Rocic P, Griendling KK (2002) NAD(P)H oxidase-derived reactive oxygen species as mediators of angiotensin II signaling. Antioxid Redox Signal 4: 899
27. Griendling KK, Minieri CA, Ollenrenshaw JD, Alexander RW (1994) Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. Circ Res 74: 1141
28. James EA, Galceran JM, Raij L (1998) Angiotensin II induces superoxide anion production by mesangial cells. Kidney Int 54: 775
29. Umekawa T, Hatanaka Y, Kurita T, Khan SR (2004) Effect of angiotensin II receptor blockage on osteopontin expression and calcium oxalate crystal deposition in the rat kidneys. J Am Soc Nephrol 15: 635
30. Toblli JE, Ferder L, Stella I, Angerosa M, Inserra F (2001) Protective role of enalapril for chronic tubulointerstitial lesions of hyperoxaluria. J Urol 166: 275
31. Umekawa T, Byer KJ, Khan SR (2004) NADPH oxidase as a source of reactive oxygen species in oxalate and calcium oxalate crystal induced injury of renal epithelial cells. In: Kidney stones: inside and out, Proceedings of the 10th International Symposium on Urolithiasis, Hong Kong, 2004, The reprographic unit, Hong Kong Polytechnic University, Hong Kong, p 118
32. Umekawa T, Byer K, Uemura H, Khan SR (2005) Diphenyleneiodium (DPI) reduces oxalate ions and calcium oxalate monohydrate and brushite crystal-induced up-regulation of MCP-1 in nrk 52e cells. Nephrol Dial Transplant (in press)
33. Rashid T, Menon M, Thamilselvan S (2004) Molecular mechanism of oxalate-induced free radical production and glutathione redox imbalance in renal epithelial cells: effect of antioxidants. Am J Nephrol 24: 557
34. Chaturvedi LS, Koul S, Sekhon A, Bhandari A, Menon M, Koul HK (2002) Oxalate selectively activates the p38 mitoge-activated protein kinase and c-Jun N-terminal kinase signal transduction pathway in renal epithelial cells. J Biol Chem 277: 13321
35. Koul HK, Menon M, Chaturvedi LS, Koul S, Sekhon A, Bhandari A, Huang M (2002) COM crystals activate the p38 mitoge-activated protein kinase (MAPK) signal transduction pathway in renal epithelial cells. J Biol Chem 277: 36845
36. Han HJ, Lim MJ, Lee YJ (2004) Oxalate inhibits renal proximal tubule cell proliferation via oxidative stress, p38 MAPK/JNK, and cPLA2 signaling pathways. Am J Physiol Cell Physiol 287: C1058
37. Jonassen JA, Cao LC, Honeyman T, Scheid CR(2003) Mechanisms mediating oxalate-induced alterations in renal cell functions. Crit Rev Eukaryot Gene Expr 13: 55
38. Antus B, Exton MS, Rosivall L (2001) Angiotensin II: a regulator of inflammation during renal disease? Int J Immunopathol Pharmacol 14: 25
39. Toblli JE, Ferder L, Angerosa M, Inserra F (1999) Effects of amlodipine on tubulointerstitial lesions in normotensive hyperoxaluric rats. Hypertension 34: 854
40. Khan SR, Kok DJ (2004) Modulators of urinary stone formation. Front Biosci 9: 1450
41. Khan SR, Byer KJ, Thamilselvan S, Hackett RL, McCormack WT, Benson NA, Vaughn KL, Erdos GW (1999) Crystal-cell interaction and apoptosis in oxalate-associated injury of renal epithelial cells. J Am Soc Nephrol 10: S457
42. Miller C, Kennington L, Cooney R, Kohjimoto Y, Cao LC, Honeyman T, Pullman J, Jonassen J, Scheid C (2000) Oxalate toxicity in renal epithelial cells: characteristics of apoptosis and necrosis. Toxicol Pharmacol 162: 132
43. Khan SR, Finlayson B, Hackett RL (1982) Experimental calcium oxalate nephrolithiasis in the rat, role of renal papilla. Am J Pathol 107: 59
44. Khan SR (1995) Experimental CaOx nephrolithiasis and the formation of human urinary stones. Scanning Microsc 9: 89
45. Fasano JM, Khan SR (2001) Intratubular crystallization of calcium oxalate in the presence of membrane vesicles: an in vitro study. Kidney Int 59: 169
46. Riese RJ, Mandel NS, Wiessner JH, Mandel GS, Becker CJ, Kleinman JC (1992) Cell polarity and CaOx crystal adherence to cultured collecting duct cells. Am J Physiol 262: F177
47. Verkoelen CF, Van der Broom BG, Houtsmuller AB, Schroeder FH, Romijn JC (1998) Increased CaOx monohydrate crystal binding to injured renal epithelial cells in culture. Am J Physiol 274: F958
48. Bigelow MW, Wienner JH, Kleinman JC, Mandel NS (1999) Surface exposure of PS increases CaOx crystal attachment to IMCD cells. Am J Physiol 272: F55
49. Wiessner JH, Hasegawa AT, Hung LY, Mandel NS (1999) Oxalate-induced exposure of PS on surface of renal epithelial cells in culture. J Am Soc Nephrol 10: S441
50. Thamilselvan S, Selvam R (1997) Effect of vitamin E and mannitol on renal calcium oxalate retention in experimental nephrolithiasis. Indian J Biochem Biophys 34: 319
51. Kumar S, Selvam R (2003) Supplementation of vitamin E and selenium prevents hyperoxaluria in experimental urolithic rats. J Nutr Biochem 14: 306
52. Selvam R, Ravichandran V (1993) Restoration of tissue antioxidants and prevention of renal stone deposition in vitamin B6 deficient rats fed with vitamin E or methionine. Indian J Exp Biol 31: 882

53. Muthukumar A, Selvam R (1998) Role of glutathione on renal mitochondrial status in hyperoxaluria. Mol Cell Biochem 185: 77
54. Ito Y, Yasui T, Okada A, Tozawa K, Hayashi Y, Kohri K (2005) Preventive effects of green tea on renal stone formation and the role of oxidative stress in nephrolithiasis. J Urol 173: 271
55. Umekawa T, Chegini N, Khan SR (2002) Oxalate ions and calcium oxalate crystals stimulate MCP-1 expression by rnal epithelial cells. Kidney Int 61: 105
56. Pearle MS (2001) Prevention of nephrolithiasis. Curr Opin Nephrol Hypertens 10: 203
57. Vaziri ND (2004) Roles of oxidative stress and antioxidant therapy in chronic kidney disease and hypertension. Curr Opin Nephrol Hypertens 13: 93
58. Griendling KK, Fitzgerald GA (2004) Oxidative stress and cardiovascular injury. Part II: animal and human studies. Circulation 108: 2034
59. Meagher EA (2003) Treatment of atherosclerosis in the new millennium: is there a role for vitamin E? Prev Cardiol Spring 6(2): 85
60. Hasnain BI, Mooradian AD (2004) Recent trials of antioxidant therapy: what should we be telling our patients? Cleve Clin J Med 71: 327
61. Baxmann AC, Mendonca COG, Heilberg IP (2003) Effect of vitamin C supplements on urinary oxalate and pH in calcium oxalate stone forming patients. Kidney Int 63: 1066